ANOPHELES VECTORS OF MALARIA IN SOUTHEAST ASIA

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The great amount of illness due to malaria among United States military forces in the Second World War evoked a great effort in the fields of chemotherapy and chemoprophylaxis resulting in the development of a number of synthetic anti-malarial drugs of great promise. Atabrine (mepacrine) was used extensively during the war, and just at the close of the conflict chloroquine was adopted for treatment of the blood stages of the malarial parasites, and as a suppressive prophylactic drug. Later chloroquine was combined with primaquine, which attacks the tissue phases of the parasites in the liver, and it appeared that the major problem of troop protection had been solved.

During the Second World War there was also an accelerated development of insecticides, culminating in the appearance of DDT and other synthetic organic compounds which were extremely effective and long lasting. In the post-war era many national governments adopted programs for malaria control or eradication with the assistance of the World Health Organization and various agencies of the United States Government. The feeling began to grow that malaria - one of the greatest scourges of mankind, and one of the major stumbling blocks to human progress in the tropics - might be on the verge of extinction.

Several events have combined to dispel this hope. It has become apparent that mosquitoes may become resistant to the insecticides upon which the control and eradication programs so largely depend. As early as 1961, sixteen important malaria vectors had been found resistant to one or more of the commonly used insecticides (9). The most recent serious obstacle to malaria eradication has been the detection of strains of malaria parasites - thus far all belonging to the dangerous species Plasmodium falciparum - which do

not respond clinically to chloroquine, nor in some cases to any of the synthetic anti-malarials. Some of these strains also show some resistance to quinine. Furthermore, the synthetic drugs do not function reliably as prophylactic agents against such strains. This problem was first detected in South America in 1961 (8), and was followed in rapid order by similar reports from Cambodia (5), Vietnam (10), Malaya (7) and Thailand (18). In late 1965 and early 1966 the problem of drug-resistant falciparum malaria became a major medical problem for U. S. military forces operating in SE Asia. This has given rise to a major new effort in drug research, and it has also given added emphasis to the equally important studies already underway in SE Asia on the distribution, vector relationships, biology and control of the mosquito vectors.

The present paper summarizes progress and current problems encountered in research on malaria and its Anopheles vectors in SE Asia, with emphasis on research in Thailand conducted by the U.S. Army Medical Component, Southeast Asia Treaty Organization (SEATO), and the Walter Reed Army Institute of Research.

The implication of a particular Anopheles species as a malaria vector depends upon the determination of a number of factors in its habits and life history. These will be discussed in some detail below in relation to one of the species investigated in Thailand, but it may be helpful to outline the major points here.

First, a determination must be made of the Anopheles species present in the area studied. The mosquitoes may be collected by a number of methods, but at least a portion should be collected as larvae and reared to the adult stage. In most areas species complexes occur which cannot readily be identified in the adult stage and require detailed taxonomic study of all stages. Some of these exceedingly similar species may have quite different feeding habits.

Secondly, the vector status of the various species must be determined by dissection of wild-caught mosquitoes and examination of the salivary glands and stomachs for presence of malaria parasites. Laboratory reared mosquitoes may also be fed on local malaria carriers to determine specific infection rates, and the rate at which parasites develop in the mosquitoes under local conditions.

A third vital element is the determination of the feeding habits of the local Anopheles, by direct collections from humans and animals, or by the use of various traps and other devices. Precipitin tests of mosquito blood meals may also be useful, but direct collection from man may provide the most readily available data.

Many other factors, such as mosquito longevity, resting habits and flight range are necessary for a complete understanding of the importance of the various species. Some of these factors become evident in obtaining the first three elements of data listed above - others may require special effort. All of these investigations are complicated by the fact that malaria is almost everywhere a sharply seasonal phenomenon, as the infection and the vectors fluctuate sharply in response to changes in rainfall, temperature and other environmental changes. In general, a period of several seasons are required before the complete vector picture for any area can be adequately understood.

In an effort to gather background data for later detailed observations, field collection of mosquitoes was begun in forested areas of Thailand in 1961; these studies are still in progress. Southeast Asia has an amazingly rich biota, including over one hundred species and subspecies of Anopheles mosquitoes. Fortunately, not all of these species are to be found in any given limited area. Through early 1966 we had identified 53 Anopheles species and subspecies from Thailand (13). We are presently examining specimens and records from Vietnam, where approximately 37 species occur, but the early data are difficult to evaluate due to confusion of scientific names in the French literature.

Only 36 of the 53 Anopheles species in Thailand have been found biting man in our collections, and some of these so rarely that they do not constitute a problem. As a rule, only one or a few species of Anopheles tend to be important malaria vectors in any given area, although small numbers of other species may be found infected from time to time. Four of the species which we collected in Thailand were known to be important vectors either in Thailand itself, or in nearby countries. These are: Anopheles minimus Theobald, A. maculatus Theobald, A. sundaicus (Rodenwaldt) and A. balabacensis Baisas.

Anopheles minimus has long been known to be an important malaria vector in the Philippines, Thailand, and many other parts of SE Asia. Its biology, and habits are well known. A. maculatus is a very important vector in Malaya, and A. sundaicus is a highly effective vector in coastal areas from Vietnam to India. The status of A. balabacensis proved to be somewhat more complex. Its importance as a malaria vector had gone largely unnoticed until McArthur (6) published a detailed outline of his own observations on the species in Borneo, and the findings of others, chiefly during the Second World War. Infection of A. balabacensis was first reported for Thailand in 1956, but it was not regarded as an important vector. Our early observations, however, plus the work of Eyles et al. (5) in Cambodia, indicated that A. balabacensis might be a very important vector in jungle areas of mainland SE Asia. Much of our attention was therefore devoted to study of this poorly understood species.

From 1963 to 1964 a field station was established on the margin of the jungle at Khao Mai Khaeo, Cholburi Province, approximately 150 K. Southeast of Bangkok (14). A very large number of A. balabacensis were collected, particularly in the rainy season (June-October) of 1963. Malaria was hyperendemic in the region, with high rates of parasitemia in adults, children and infants, and a high spleen index.

A. balabacensis was found to feed avidly on man, indoors as well as outdoors, particularly during the hours between midnight and dawn. Smaller numbers A. minimus, A. maculatus and several other species were collected by a number of methods (table 1). By the end of the study over twenty species were collected within a few kilometers of the field station. Most of the collections were made by teams of four local employees, each with a professional supervisor, working from dusk to dawn. Collections were made from sleeping and active local inhabitants, their livestock, dwellings and surrounding vegetation. In one ten-night period over 11,000 A. balabacensis were collected, permitting an accurate assessment of its habits. It proved to be extremely anthropophilic, attacking man chiefly in the hours after midnight, but in limited numbers from dusk to dawn. It was not at all attracted to cattle in the area (table 1). After feeding, the females moved from the vicinity of the dwellings and by mid-morning all had returned to the jungle from which they had come. The only breeding sites discovered were small pools of water in the sandy soil under cover of the neighboring jungle. The feeding and resting habits of A. balabacensis seemed to fit it particularly well as a vector of malaria, and also indicated that it might be a particularly difficult vector to control by the usual method of house spraying.

Large numbers of A. balabacensis, A. minimus and A. maculatus were dissected but only the first two were found with malaria parasites. In one series, 8.7 per cent of the A. balabacensis and 2.5 per cent of the A. minimus females had sporozoites in the salivary glands. The efficiency of A. balabacensis as a vector was confirmed by feeding reared specimens on patients with vivax and falciparum malaria infections. A large percentage of the A. balabacensis reached the infective stage with P. vivax in 11-12 days, and with P. falciparum in 14-18 days at a laboratory temperature of 78-82°F. Large numbers of A. balabacensis survived for over 30 days in the laboratory under these conditions. Parallel feedings by A. minimus and A. maculatus were not successful, either because of failure of the mosquitoes to feed, or their death before the parasites reached the infective stage.

The large numbers of A. balabacensis collected biting man at Khao Mai Khaeo and the high rate of infection made it probable that susceptible individuals might become infected after spending as little as one night in the area. This proved to be the

Table 1. Comparative *Anopheles* mosquito captures, July to September 1963. Khao Mai Khaeo, Cholburi Province, Thailand (38 nights)

Species	Biting man	Biting cattle	Other methods	Total
An. aconitus	17	33	9	59
" balabacensis	71	--	2004	2075
" barbirostris	2	2	104	108
" hyrancus' group	--	25	--	25
" jamesii	--	247	--	247
" karwari	--	1	--	1
" kochi	1	27	--	28
" maculatus	89	4	229	322
" minimus	9	--	98	107
" philippinensis	10	41	2	53
" splendidus	6	23	1	30
" tessellatus	2	1	--	3
" vagus	10	36	2	48
TOTAL	217	440	2449	3106

case for two Thais and two U.S. personnel. Actually, all of those participating in the study over a two year period contracted falciparum malaria at least once despite the rigid enforcement of drug prophylaxis. It was apparent that many of the P. falciparum infections in the area were drug-resistant, and these infections did, in fact, generally fail to respond to any of the synthetic drugs. It was later demonstrated that large populations of A. balabacensis were not required for transmission. In May, 1965 a party of five entered the Khao Mai Khaeo area in search of an area for insecticide tests. The mosquito population was extremely low due to delayed onset of the monsoons and a heavy DDT application which had been made by the Thai Ministry of Public Health teams the previous week. Four experienced collectors in the party could find only six Anopheles from dusk to dawn, but two individuals contracted falciparum infections despite chloroquine-primaquine prophylaxis. In September 1965, a party of four were selecting sites for mosquito repellent tests in the jungle northeast of Bangkok along a road being constructed by U. S. Army Engineer troops. These four collectors could find only 14 A. balabacensis, and no other vectors, in two nights, but two members of the party contracted falciparum malaria, again despite chloroquine-primaquine prophylaxis.

Thus, this species appears to be a highly effective vector in forested areas. Houses in the primary study area at Khao Mai Khaeo as far as a half-mile from the jungle had high biting rates, and it is assumed that this mosquito may fly as far as a mile in search of a blood meal. Attempts at tracing mosquitoes with use of metallic-dust markers failed due to the extreme difficulty in finding the adults in their daytime resting places in the jungle. However, most of its biting activity appears to be restricted to the forest and the forest margins. The species is strongly associated with tropical evergreen and monsoon forests and a rough approximation of its range may be judged from the extent of such forests in SE Asia, (figure 1). This includes upland regions of Vietnam, and the species has been implicated there as a vector, along with A. minimus, A. jeyporiensis, and several other species. Since infections in U.S. military personnel in Vietnam are occurring chiefly in combat forces in action in the jungle there appears to be a high degree of probability that many infections are being transmitted by A. balabacensis. The seriousness of this problem for the U. S. Army may be seen from the reports of combat actions in November and December, 1965 in which malaria attack rates exceeded combat losses, and approached or exceeded the rates seen in the island campaigns in the Pacific area in the Second World War (15). A large proportion of these cases appear to be refractory to treatment with the standard synthetic anti-malarial drugs, and are not always clinically cured with large doses of quinine. At present the rigid adherence to malaria discipline, including the

use of repellent and the use of bed nets where possible, offer the best protection under jungle conditions. The type of combat in which the troops are presently engaged will make mosquito control by insecticide or environmental measures difficult.

All of the well-documented cases of chloroquine-resistant falciparum malaria from Malaya, Cambodia, Thailand and Vietnam have occurred within the range of A. balabacensis, and it has been suggested that there may be some relationship (12). To date, no resistance has been reported from areas of Malaya where A. maculatus is the principal vector, nor from coastal areas of SE Asia where A. sundaicus is the principal vector. There is no reason at present to suspect that one mosquito species may transmit drug-resistant strains of malaria parasites selectively. On the other hand, the marked overlap of distribution of drug-resistant falciparum malaria, tropical evergreen and monsoon forest and the range of A. balabacensis is suggestive and requires additional study in the field and laboratory.

In Thailand, A. minimus occurs from the forest margins outward into more open agricultural land. It breeds in open rivulets and irrigation ditches and tends to rest in houses during the day. Thus its control is far simpler than control of A. balabacensis. Both species occur over much of upland SE Asia, but in South Thailand they extend only to the general area of the Malayan border. At that point A. balabacensis is replaced by a bewildering array of at least five closely related forms which are still under study by our cooperators at the U. S. National Museum and the British Museum. Typical A. balabacensis extends into the far Northwest tip of Malaya, and it may be significant that this is the only place in Malaya where well-documented drug-resistant malaria has been detected (7).

Anopheles sundaicus is an important malaria vector in coastal areas of SE Asia, including Vietnam and Thailand (11). It may become extremely abundant in pools of brackish water at times, causing local epidemics of malaria. In late 1965 we collected this species in coastal southeastern Thailand in association with A. balabacensis in an area where heavily wooded hills approach the coast. When this area was visited in 1963 no A. sundaicus were collected and while A. balabacensis were present they did not appear to be attracted to man. There was little or no malaria in the area in 1963, but a significant amount was found in 1965. Furthermore, A. balabacensis was now attacking man in some numbers, particularly in the early evening hours, whereas elsewhere in Thailand it characteristically feeds after midnight.

Anopheles maculatus illustrated another type of behavioral or physiological variation which makes malaria investigations

complicated and keyed to particular areas. In Malaya it is a very important vector, but elsewhere in SE Asia, while it has been found carrying malaria parasites from time to time, it does not appear to be important. It is very abundant in parts of Thailand, but we have found no infected specimens. Examination of specimens from Malaya, Thailand, Laos, Burma, Cambodia and Vietnam has failed to disclose any morphological variation. It is possible that the species is a complex, some members of which differ in feeding behavior, genetics and physiology, but that these differences have not reached the level of morphological differentiation. Detailed analysis by Davidson and his associates in London (3) have shown this to be the case in the Anopheles gambiae complex of Africa. There is no reason to suppose that once more sophisticated studies have been made these distinctions will not also be found in Anopheles species in SE Asia.

A further complication in the epidemiology of malaria in SE Asia was introduced in 1960 by the finding that a vivax-like simian parasite (Plasmodium cynomolgi, B type) could be transmitted to man by mosquito bite (4). Chin and his associates (2) recently reported a natural infection of a U. S. Army Map Service surveyor in Malaya with Plasmodium knowlesi, a monkey parasite resembling P. malariae of man. The significance of these observations for military units operating in jungle areas is obvious, but much additional study is required. Large primate populations exist in many jungle areas of SE Asia, sometimes in close proximity to man. We have found (table 2) that both A. balabacensis and A. minimus fed readily on macaque monkeys and man. Cheong and his associates (1) have recently isolated two simian parasites from A. balabacensis in northwestern Malaya in an area where this species feeds on man as well, and the findings of Wharton and his associates (17) indicate that other mosquito species may also feed on man and monkeys.

Table 2. Anopheles ♀♀ attracted to human and monkey bait in net traps at ground level. Khao Mai Kaeo. (After Scanlon and Sandhinand, 1965)

Species	Females per trap night	
	Human (10 nights)	Monkeys (3 nights)
A. balabacensis	14.6	32.3
A. minimus	0.2	1.0
A. maculatus	2.8	4.3
A. barbirostris	1.1	0.3
A. hyrcanus group	0.4	--
A. vagus	0.3	--
A. philippinensis	0.2	--
A. splendidus	0.1	--

We have also begun the evaluation of the gibbon, Hylobates lar, a species more closely allied to man than the macaques, both as a source of human infection and as an experimental animal for studies of falciparum malaria. Preliminary investigations (16) indicate that the splenectomized gibbon is fully susceptible to blood-induced falciparum malaria and infections are maintained for three months or longer. Both asexual parasites and gametocytes are produced at relatively high densities (Figure 2). Animals with intact spleens can be infected, but only for short periods of time. Currently studies are underway to demonstrate mosquito transmission of falciparum parasites to these primates. Gibbons are quite common in forested areas of SE Asia, but their status as a source of human infection in the wild is still under study.

SUMMARY

The detection of strains of Plasmodium falciparum in SE Asia which are resistant to chloroquine and other synthetic antimalarial drugs has intensified research on all phases of malaria, including study of the Anopheles fauna of the area. In late 1965 the U. S. Army experienced a severe outbreak of malaria among troops operating in forested areas of Vietnam which were very similar to jungle habitats which have been investigated by entomologists of the U. S. Medical Research and Development Command in neighboring Thailand since 1963. In the latter areas, and presumably in much of Vietnam, Anopheles minimus and A. balabacensis are the most important vectors. The latter species is a particularly effective malaria vector in forested areas in much of SE Asia, but its habits were poorly known until recently. It is highly anthropophilic and bites freely in the jungle. Almost all of the personnel engaged in the investigations in Thailand contracted falciparum malaria despite prophylactic drugs and other protective measures. The vector status of A. sundaicus and A. maculatus was also investigated in Thailand, and these species and others may also be involved in malaria transmission in Vietnam.

Recent observations in SE Asia suggest that monkeys and other primates may serve as sources of malaria infection for humans in the forests. Anopheles balabacensis feeds readily on monkeys in forested areas and might serve to carry infections to man under some circumstances. It has been possible to transmit human falciparum malaria to the gibbon, an anthropoid ape common in the jungles, by blood inoculation, and this primate is also under study as a source of human infection.

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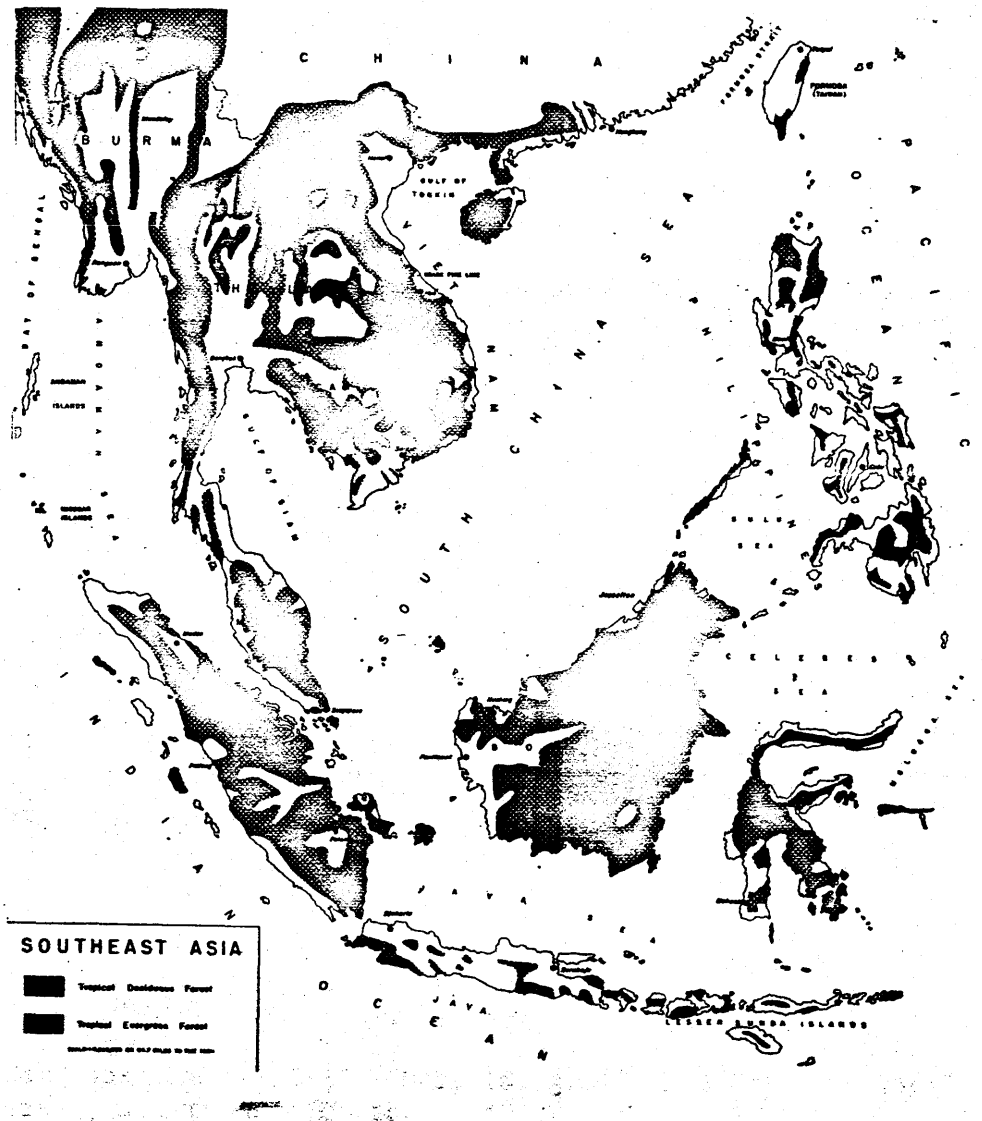

Figure 1. Distribution of Tropical Deciduous Forest and Tropical Evergreen Forest in Southeast Asia.

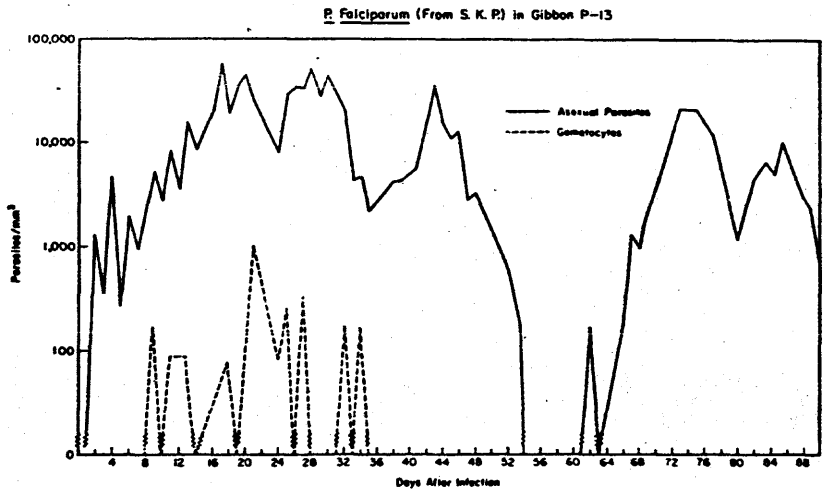

Figure 2. Response of the splenectomized gibbon, Hylobates lar, to blood-induced infection with Plasmodium falciparum.